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From: Gabel, Gailene  
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Subject: 09/817,448

Please provide a copy of the following literature ASAP:

- 1) SERAFINI A.N. "Iodine-123-Rose Bengal: An Improved Hepatobiliary Imaging Agent", Journal of Nuclear Medicine, vol. 16, no. 7 (1975), pp. 629-632.
- 2) NECKERS D.C. "Rose Bengal", Journal of Photochemistry and Photobiology. A Chemistry (1989) vol. 47, pp. 1-29.
- 3) NORMAN et al. (Invest Radiol, 26: S120-S121, 1991).

Thanks a bunch,  
Gailene R. Gabel  
7B15  
305-0807

## REVIEW

# ROSE BENGAL<sup>†</sup>

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(Received August 12, 1988)

## Summary

The spectral properties, photochemical reactivity and photophysical parameters of all of the known derivatives of Rose Bengal, 2,4,5,7-tetraiodo-3,4',5',6'-tetrachlorofluorescein, are reported and compared.

## 1. Background

Rose Bengal, 2,4,5,7-tetraiodo-3,4',5',6'-tetrachlorofluorescein [1], was originally synthesized by Gnehm [2] as a fabric dye to mimic the red colors in "bengalis" [3]. Its name is connected to the red symbolic spot worn at the part of the hair by Bengali women to symbolize marriage [4]. Much of the history of Rose Bengal has been reviewed elsewhere [5, 6].

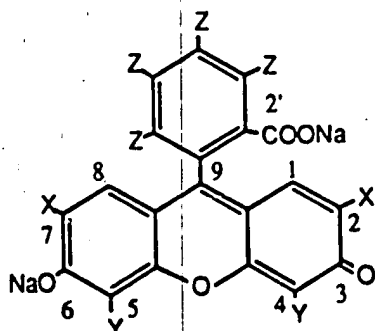
Rose Bengal, disodium salt, holds an important position among all dyes for a number of reasons:

1. It has a large absorption in all solvents in which it is soluble which corresponds almost exactly to the sodium D line.
2. Its intersystem crossing yield is high, but  $\Phi_{isc}$  is not unity. All Rose Bengals show some fluorescence.
3. Its triplet is completely quenched by oxygen.
4. Its spectrum is most diagnostic of its immediate environment.
5. It bleaches slowly in protic, polar solvents.
6. It is a photodynamic sensitizer.
7. Its singlet may be quenched by strong oxidizing agents, in some cases forming radicals.

The purpose of this review is to summarize the spectral, photophysical, and photochemical properties of Rose Bengal, disodium salt, as well as those of its many derivatives. We refer to the disodium salt of 2,4,5,7-tetraiodo-3,4',5',6'-tetrachlorofluorescein as Rose Bengal since this was Gnehm's original dyestuff and it is what is sold commercially as Rose Bengal.

<sup>†</sup>Contribution 28 from the Center for Photochemical Sciences.

TABLE 1  
The xanthenes



Compound	X	Y	Z	$\lambda_{\max}$ (nm) in $H_2O$	$\lambda_{\max}$ (nm) in EtOH	$\phi(EtOH)^a$	$\phi(H_2O)^a$	$pK_1^b$	$pK_2$
Rose Bengal	I	I	Cl	549.0	557.0	0.86	0.75	3.72	—
Phloxin	Br	Br	Cl	538.5	547.5	0.40	0.65	3.69	—
Erythrosin	I	I	H	526.5	532	0.69	0.63	4.18	—
Eosin	Br	Br	H	517.0	523	0.32	0.57	3.80	—
	Cl	Cl	H	510.0	520	0.05	0.05	—	—
	I	H	H	506.5	513	0.33	0.48	3.75	4.75
	Br	H	H	504	510	0.32	0.42	—	—
	H	Cl	H	502.5	511	0.04	0.07	—	—
	Cl	H	H	502.5	511	0.04	0.07	3.69	4.91
Fluorescein	H	H	H	490.0	499	0.03	0.03	4.38	6.99

Most of the data in this table is from E. Gandin, Y. Lion and A. Van de Vorst, *Photochem. Photobiol.*, 37 (1983) 271.

<sup>a</sup>Singlet oxygen formation.

<sup>b</sup>Data from I. M. Issa, R. M. Issa and M. M. Ghoneim, *Z. Phys. Chem.*, 250 (1922) 161.

For many of the other xanthenes, Eosin and fluorescein in particular, ring-substituted derivatives are also referred to as Eosins or fluoresceins respectively (for example Eosin B or fluorescein yellow). In general these compounds have the same ring skeleton as the parent compound but completely different ring substituents. To avoid confusion we will draw structures where necessary. Though we will generally avoid detailed comparisons with the xanthenes as a group, comparisons with fluorescein, Erythrosin and Eosin will be made where appropriate.

## 2. Analogs and synthesis

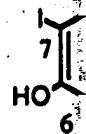
The structures and simple photophysical properties of the dyes in water and EtOH are shown in Table 1. The synthesis of the xanthenes is from resorcinol and a phthalic anhydride, in the case of Rose Bengal tetrachlorophthalic anhydride. It is trivial but messy.



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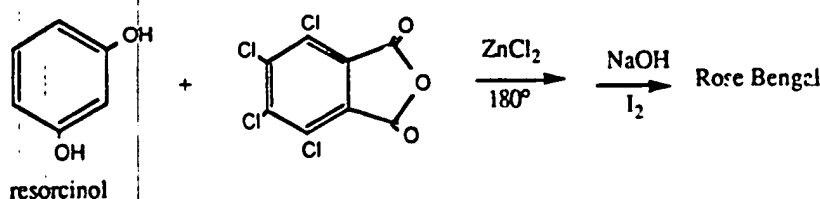
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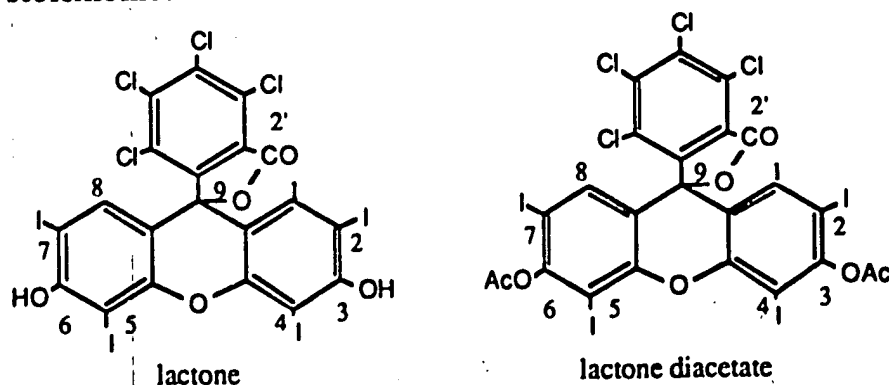
3. A

tion



As a dye neither Rose Bengal nor any of its derivatives has current commercial application though Erythrosin is used as the red dye in marachino cherries and Tylenol<sup>®</sup> capsules. Eosin has many biological uses, as a biological stain for eosinophiles, for example. The uses of fluorescein derivatives are unique and extremely important. It is used as a diagnostic probe in many clinical applications, and in fact there is even a company which markets such compounds called Molecular Probes. Unfortunately these applications are extensive and would require much more space than is allowed for this review. We will confine our report to the chemistry of the Rose Bengals and discuss biological issues in a subsequent review.

The disodium salt of Rose Bengal is difficult to purify and the commercially available dyes are not seriously purified after synthesis. Rose Bengal frequently contains inorganic salts either derived during the synthesis or added as part of an old farbstoff protocol. Dye purity is reported relative to Colour Index methods [7, 8] which are often old [5]. Rose Bengal has been reportedly purified by chromatography [9] though these methods often accomplish little other than making the laboratory table a big red mess. Rose Bengal is best purified by conversion to the lactone [10] or to the lactone diacetate [11], with subsequent recrystallization and stoichiometric reconversion to the disodium salt.



The C-2',C-6 ammonium salts [10], phosphonium salts, iodonium salts and other organic derivatives are easily prepared from the lactone by neutralization with the appropriate Lewis base. These organic ion salts are much easier to purify though they are not, for the most part, soluble in water.

### 3. Absorption and emission spectra

Rose Bengals retain their general xanthene color and spectra if ionization at C-6 is supported by the solvent. This depends on a number of factors,

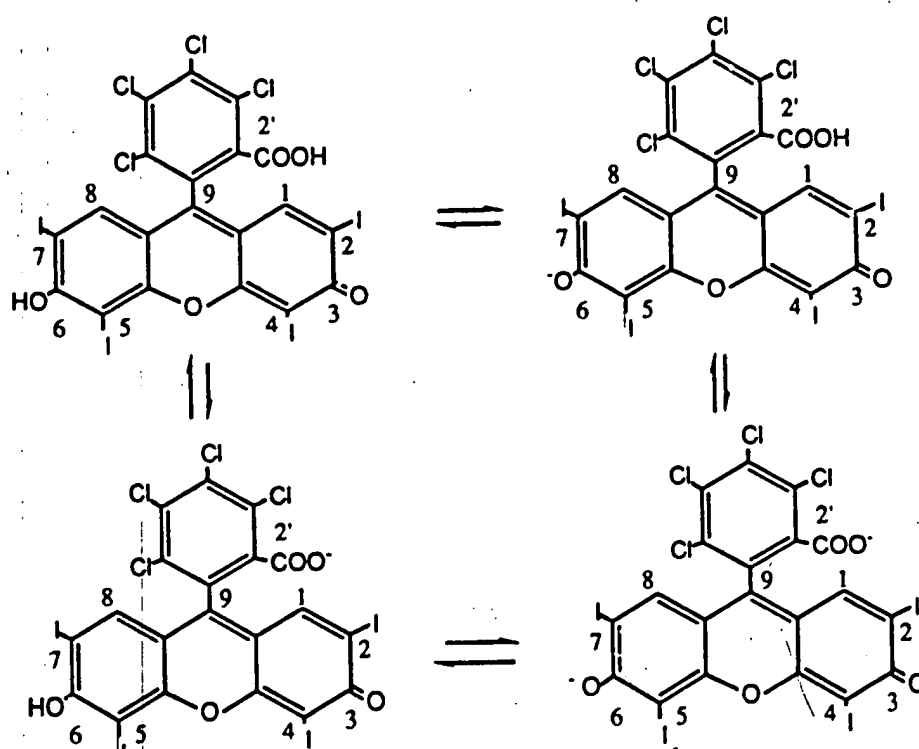


Fig. 1. Top left, C-2'(H),C-6(H); top right, C-6 monoanion; bottom left, C-2' monoanion; C-6(H); bottom right, dianion.

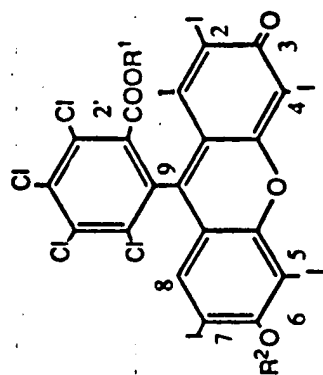
among which is the solubility of the gegen ion in the supporting solvent. The essential equilibria are shown in Fig. 1. The important forms of Rose Bengal and all of the xanthenes are the C-6 monoanion (Fig. 1, top right) and C-2',C-6 dianion (Fig. 1, bottom right). The fully protonated C-2'(H), C-6(H) (Fig. 1, top left) has never been observed since it undergoes an immediate ring closure to the lactone. The  $pK_1$  of the C-6 protio form of Rose Bengal has been measured in water as 3.72. This contrasts with a value for the ionization constant for the  $pK_2$  at C-2' of 4.75 for diiodofluorescein (Table 1). The ionization constant at C-2' in Rose Bengal has not been (or cannot be) measured because ring closure occurs to the lactone immediately upon formation of the C-2' acid.

Essentially three different kinds of Rose Bengal derivatives have been synthesized. Salts with gegen ions other than sodium at both C-2' and C-6, C-2' ester, C-6 salts, and C-2' ester, C-6 ethers. The absorption spectra of all the known Rose Bengal dianionic and C-2' ester, C-6 monoanions are given in Table 2. The emission spectra of known Rose Bengal derivatives are given in Table 3.

Four variables cause significant changes in the spectrum of the dye: (a) C-6 effects and ionization at C-6 [12]; (b) C-2' substitution; (c) solvent and solvent composition and (d) concentration. We discuss each of these issues separately below.

TABLE 2

Absorption properties of known derivatives of Rose Bengal



Compound	Water†	MeOH	EtOH	CH <sub>2</sub> Cl <sub>2</sub>	DME	MeTHF	Other
R <sup>1</sup> = R <sup>2</sup> = Na <sup>+</sup>	515	549	518	558	515	557	n-PrOH (559) <sup>a</sup> n-BuOH (561) <sup>a</sup>
Li <sup>+</sup> ; Li <sup>+</sup> b	—	—	519	559	518	557	—
K <sup>+</sup> ; K <sup>+</sup> b	—	—	520	558	—	—	—
Cs <sup>+</sup> ; Cs <sup>+</sup> b	—	—	519	559	—	—	—
φ <sub>3</sub> I <sup>+</sup> ; φ <sub>3</sub> I <sup>+</sup> c	—	—	519	558	522	566	524
φ <sub>3</sub> P <sup>+</sup> CH <sub>3</sub> φ <sub>3</sub> P <sup>+</sup> CH <sub>3</sub> c	—	—	518	559	(37500)	(102200)	(33000)
(CH <sub>3</sub> ) <sub>3</sub> NH <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub> NH <sup>+</sup> b	—	—	519.5	559	529	564	520
(Et) <sub>3</sub> NH <sup>+</sup> (Et) <sub>3</sub> NH <sup>+</sup> b	—	—	521	559	(29700)	(114200)	(29500)
	—	—	(3800)	(117000)	523	557	563
	—	—	(33200)	(117000)	(27600)	(59400)	(110700)
	—	—	—	—	519	558	—
	—	—	—	—	(44900)	(101500)	—

(continued)

TABLE 2. (continued)

Compound	Water <sup>†</sup>	MeOH	EtOH	CH <sub>2</sub> Cl <sub>2</sub>	DME	MeTHF	Other
(Et) <sub>2</sub> NH <sub>2</sub> <sup>+</sup> (Et) <sub>2</sub> NH <sub>2</sub> <sup>+</sup> <sup>b</sup>	-	-	520 (29100)	527 <sup>†</sup>	553	-	-
piperidine; piperidine <sup>b</sup>	-	-	521 (43700)	526 <sup>†</sup>	553	-	-
R <sup>1</sup> = CH <sub>3</sub> ; R <sup>2</sup> = Na <sup>+</sup> 548 (113600)	-	-	527 (37100)	-	-	-	-
R <sup>1</sup> = C <sub>2</sub> H <sub>5</sub> ; R <sup>2</sup> = Na <sup>+</sup>	-	-	524 (29600)	-	-	-	-
R <sup>1</sup> = CH <sub>2</sub> Φ; R <sup>2</sup> = Na <sup>+</sup> <sup>b,d</sup>	-	-	527 (33800)	-	528 (39300)	569 (99400)	-
R <sup>1</sup> = CH <sub>3</sub> ; R <sup>2</sup> = H <sup>+</sup> <sup>b</sup>	-	-	526 (40900)	-	-	-	-
R <sup>1</sup> = CH <sub>2</sub> Φ; R <sup>2</sup> = TMA <sup>+</sup> <sup>b</sup>	-	-	527 (26000)	527 (30600)	566 (45700)	-	-
R <sup>1</sup> = CH <sub>2</sub> Φ; R <sup>2</sup> = TEA <sup>+</sup> <sup>b</sup>	-	-	527 (36200)	527 (24600)	564 (44300)	-	-
R <sup>1</sup> = CH <sub>2</sub> Φ; R <sup>2</sup> = DEA <sup>+</sup> <sup>b</sup>	-	-	527 (38500)	530 (35500)	561 (47600)	-	-
R <sup>1</sup> = octyl; R <sup>2</sup> = (Bu) <sub>4</sub> N <sup>+</sup> <sup>e</sup>	-	525 564	524 (24 500)	-	-	-	toluene 536 563
R <sup>1</sup> = octyl; R <sup>2</sup> = (Bu) <sub>3</sub> NH <sup>+</sup>	-	-	488 (3500)	567 (128000)	-	-	toluene 536 563 537 (34000) 585 (65000) <sup>g</sup>
R <sup>1</sup> = ethyl; R <sup>2</sup> = Et <sub>3</sub> NH <sup>+</sup> <sup>e</sup>	-	524 563	-	525	563	-	-

R<sup>1</sup> = ethyl;R<sup>2</sup> = Bu<sub>4</sub>N<sup>+</sup><sup>e</sup>R<sup>1</sup> = CH<sub>2</sub>Φ;R<sup>2</sup> = piper<sup>+</sup><sup>b</sup>

527

566

565.7

532

574

570

570

570

570



$R^1 = \text{ethyl};$ $R^2 = \text{Et}_3\text{NH}^+e$	524	563	525	563	570
$R^1 = \text{ethyl};$ $R^2 = \text{Bu}_4\text{N}^+e$	523	563	532	574	toluene 537 570
$R^1 = \text{CH}_2\phi;$ $R^2 = \text{piper}^+b$	528 (38400)	565.7 (94400)	526.5 (41000)	565.7 (42700)	—
$R^1 = \text{CH}_2\phi;$ $R^2 = \phi_1^+e$	526 (38300)	568 (119000)	530 (38100)	572 (92600)	toluene 540 (39400)
$R^1 = \text{CH}_2\phi;$ $R^2 = \phi_1\text{PCH}_3^+e$	525 (36100)	567 (118000)	530 (32700)	573 (118000)	toluene 534 (27500)
$R^1 = \text{CH}_2\phi;$ $R^2 = \text{triphenyl}$ $\text{pyrillium}^+c$	527 (27500)	568 (864000)	528 (20800)	572 (17800)	toluene 526†
$R^1 = \text{CH}_2\phi;$ $R^2 = \phi_1\text{SCH}_3^+e$	525 (26300)	566 (80200)	529 (27600)	572 (92000)	toluene 530 (7300)
$R^1 = \text{Z-Lys};$ $\text{TEA}^b$	525 (11670)	564 (33400)	—	—	—
$R^1 = \text{Z-(Lys)}_4\text{-OMe};$ $\text{TEA}^+b$	525 (11740)	565 (33500)	—	—	—
$R^1 = \text{-(CH}_2)_5\text{CO}_2^-$ $R^2 = \text{Na}^+e$	551.5 (82100)	—	—	—	—
$R^1 = \text{-(CH}_2)_5\text{CO}_2^-$ $R^2 = \text{Na}^+f$	557 (79200)	—	—	—	—
$R^1 = \text{-(CH}_2)_5\text{CO}_2\beta\text{-cyclodextrin}$ $R^2 = \text{Na}^+$	562 (30200)	—	—	—	—
$R^1 = \text{-(CH}_2)_5;$ $R^2 = \text{Na}^+, \text{Na}^+$	5.7 (66000)	565 (183400)	—	—	515 (46100)
$R^1 = \text{-(CH}_2)_5;$ $R^2 = \text{Na}^+, \text{Na}^+$	480 (12300)	526 (63200)	565 (190200)	—	547s (78900)
					510 (26700)
					546s (48400)

(continued)

TABLE 2 (continued)

Compound	Water <sup>a</sup>	MeOH	EtOH	CH <sub>2</sub> Cl <sub>2</sub>	DME	MeTHF	Other
R <sup>1</sup> = -(CH <sub>2</sub> ) <sub>10</sub> ; R <sup>2</sup> = Na <sup>+</sup> , Na <sup>+</sup>	-	-	485 (13200)	520 (61100)	565 (20000)	-	511 (50400) 544 (98800)
R <sup>1</sup> = -(CH <sub>2</sub> ) <sub>5</sub> ; R <sup>2</sup> = NBu <sub>4</sub> <sup>+</sup> ; NBu <sub>4</sub> <sup>+</sup>	-	-	483 (12900)	523 (57900)	561 (16800)	-	-
R <sup>1</sup> = polystyrene; R <sup>2</sup> = Na <sup>+</sup>	-	-	-	-	523 562	-	529 534 562 <sup>i</sup> 562 <sup>j</sup>

<sup>a</sup> All entries  $\lambda_{\max}$  ( $\epsilon$  (l<sup>-1</sup> m<sup>-1</sup>)).

<sup>b</sup> G. O. Schenck and K. Gollnick, *Forsch. Landes Nordrhein-Westfalen*, Nr. 1256, Köln und Opladen 1963.

<sup>c</sup> J. Paczkowski, J. J. M. Lamberts, B. Paczkowska and D. C. Neckers, *J. Free Radicals Biol. Med.*, 1 (1985) 341.

<sup>d</sup> S. M. Linden and D. C. Neckers, *Photochem. Photobiol.*, 47 (1988) 543.

<sup>e</sup> The compound is not very soluble in CH<sub>2</sub>Cl<sub>2</sub>.

<sup>f</sup> J. J. M. Lamberts and D. C. Neckers, *Z. Naturforsch., Teil B*, 39 (1983) 474.

<sup>g</sup> D. Luttrull, O. Valdes-Aguilera, S. M. Linden, J. Paczkowski and D. C. Neckers, *Photochem. Photobiol.*, 47 (1988) 551.

<sup>h</sup> D. C. Neckers and J. Paczkowski, *Tetrahedron*, 42 (1986) 4671.

<sup>i</sup> 95% water/5% EtOH.

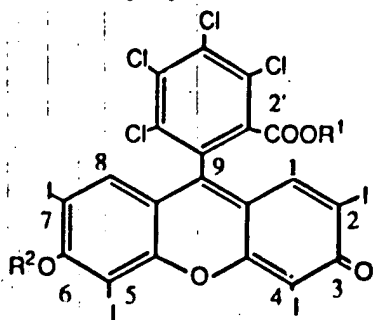
<sup>j</sup> DMF.

<sup>k</sup> Toluene.

<sup>l</sup> Cyclohexane.

TABLE 3

Emission properties of known derivatives of Rose Bengal



Compound		Water <sup>a</sup> ( $\phi$ )	MeOH	EtOH	MeTHF	Other
R <sup>1</sup> = Na <sup>+</sup> ; R <sup>2</sup> = Na <sup>+</sup>	Fl(R.T.) <sup>b</sup>	— (0.01)	573 (0.08)	563 (0.11)	587	n-PrOH (0.13)
	Fl(77 K)	—	—	569 620	—	n-BuOH (0.14) <sup>c</sup>
	Ph(77 K) <sup>d</sup>	—	702	717	723	—
R <sup>1</sup> = Li <sup>+</sup> ; R <sup>2</sup> = Li <sup>+</sup>	Fl(R.T.)	—	572	—	—	—
	Fl(77 K)	—	—	568 620	—	—
	Ph(77 K)	—	—	716	—	—
R <sup>1</sup> = K <sup>+</sup> ; R <sup>2</sup> = K <sup>+</sup>	Fl(R.T.)	—	572	—	—	—
	Fl(77 K)	—	—	569 620	—	—
	Ph(77 K)	—	—	713	—	—
R <sup>1</sup> = Cs <sup>+</sup> ; R <sup>2</sup> = Cs <sup>+</sup>	Fl(R.T.)	—	573	—	—	—
	Fl(77 K)	—	—	571 620	—	—
	Ph(77 K)	—	—	712	—	—
R <sup>1</sup> = TMA <sup>+</sup> ; R <sup>2</sup> = TMA <sup>+</sup>	Fl(R.T.)	—	573	—	—	—
	Fl(77 K)	—	— 528	589 610	—	—
	Ph(77 K)	—	—	710	—	—
R <sup>1</sup> = DEA <sup>+</sup> ; R <sup>2</sup> = DEA <sup>+</sup>	Fl(R.T.)	—	573	—	—	—
	Fl(77 K)	—	— 532	569 618	—	—
	Ph(77 K)	—	—	713-15	—	—
R <sup>1</sup> = TEA <sup>+</sup> ; R <sup>2</sup> = TEA <sup>+</sup>	Fl(R.T.)	—	573	—	—	—
	Fl(77 K)	—	— 530	569 617	—	—
	Ph(77 K)	—	—	710-11	—	—
R <sup>1</sup> = pip <sup>+</sup> ; R <sup>2</sup> = pip <sup>+</sup>	Fl(R.T.)	—	571	—	—	—
	Fl(77 K)	—	— 531	568 617	—	—
	Ph(77 K)	—	—	708-10	—	—
R <sup>1</sup> = CH <sub>3</sub> ; R <sup>2</sup> = Na <sup>+</sup>	Fl(R.T.)	—	583-4	—	—	—
	Fl(77 K)	—	— —	571 620	—	—
	Ph(77 K)	—	—	712-13	—	—
R <sup>1</sup> = CH <sub>3</sub> ; R <sup>2</sup> = H <sup>+</sup>	Fl(R.T.)	—	581	—	—	—
	Fl(77 K)	—	— 535	571 624	—	—
	Ph(77 K)	—	—	715	—	—
R <sup>1</sup> = CH <sub>2</sub> $\phi$ ; R <sup>2</sup> = Na <sup>+</sup>	Fl(R.T.)	—	584-5	—	—	—
	Fl(77 K)	—	— —	573 625	—	—
	Ph(77 K)	—	—	710	—	—
R <sup>1</sup> = CH <sub>2</sub> $\phi$ ; R <sup>2</sup> = H <sup>+</sup>	Fl(R.T.)	—	581	—	—	—
	Fl(77 K)	—	— 535	576 622-3	—	—
	Ph(77 K)	—	—	695-6	—	—

(continued)

TABLE 3 (continued)

Compound		Water <sup>a</sup> ( $\phi$ )	MeOH	EtOH	MeTHF	Other
R <sup>1</sup> = CH <sub>2</sub> $\phi$ ; R <sup>2</sup> = TMA <sup>+</sup>	Fl(R.T.)	—	—	—	—	—
	Fl(77 K)	—	—	533	576 618	—
	Ph(77 K)	—	—	—	704	—
R <sup>1</sup> = CH <sub>2</sub> $\phi$ ; R <sup>2</sup> = TEA <sup>+</sup>	Fl(R.T.)	—	—	—	—	—
	Fl(77 K)	—	—	534	575 618	—
	Ph(77 K)	—	—	—	713	—
R <sup>1</sup> = CH <sub>2</sub> $\phi$ ; R <sup>2</sup> = DEA <sup>+</sup>	Fl(R.T.)	—	—	—	—	—
	Fl(77 K)	—	—	533	576 618	—
	Ph(77 K)	—	—	—	700-1	—
R <sup>1</sup> = CH <sub>2</sub> $\phi$ ; R <sup>2</sup> = pip <sup>+</sup>	Fl(R.T.)	—	—	—	—	—
	Fl(77 K)	—	—	534-5	576 618	—
	Ph(77 K)	—	—	—	702	—
R <sup>1</sup> = ethyl; R <sup>2</sup> = TEA <sup>+</sup>	Fl(R.T.)	—	585	590	—	—
	Fl(77 K)	—	—	586 625	—	—
	Ph(77 K)	—	—	710	—	—
R <sup>1</sup> = ethyl; R <sup>2</sup> = Bu <sub>4</sub> N <sup>+</sup>	Fl(R.T.)	—	592	600	—	—
	Fl(77 K)	—	—	585 623	—	—
	Ph(77 K)	—	—	708	754	—
R <sup>1</sup> = Z-Lys; R <sup>2</sup> = TEA <sup>+</sup>	Fl(R.T.)	—	588	—	—	—
	Fl(77 K)	—	—	577 625	—	—
	Ph(77 K)	—	—	719	—	—
R <sup>1</sup> = Z-[Lys]-OMe; R <sup>2</sup> = TEA <sup>+</sup>	Fl(R.T.)	—	588	—	—	—
	Fl(77 K)	—	—	580-1 625	—	—
	Ph(77 K)	—	—	720-1	—	—

<sup>a</sup>All phosphorescence spectra were measured at 77 K unless otherwise indicated.

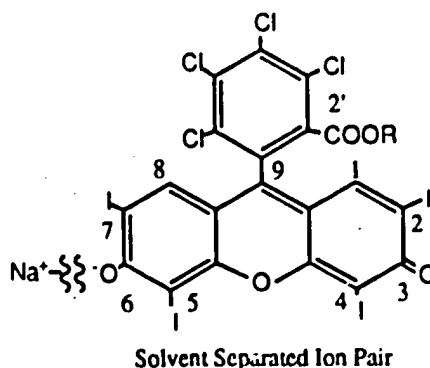
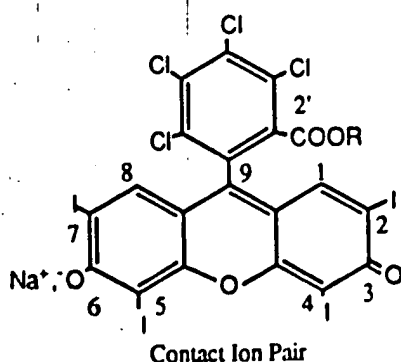
<sup>b</sup> $\lambda_{ex}$  = 500 nm unless otherwise noted.

<sup>c</sup>20 °C.

<sup>d</sup> $\lambda_{ex}$  = 550 nm unless otherwise noted.

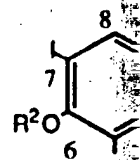
### 3.1. Substitution at C-6

Because of the sterically imposing *ortho* iodines at C-6, the organic reactivity of Rose Bengal, disodium salt, as a nucleophile is entirely at the C-2' position and most of the known Rose Bengal derivatives which have been prepared take advantage of this reactivity. Unionized derivatives at C-6 show a typical spectrum regardless of whether the spectra are of covalent derivatives or of contact ions pairs.



This at C-6 and 404 important the special special compared, sp C-2' ester is an ex and C-6 of pure substitut carboxyl (5850), (5930) of the ti Table 4.

TABLE 4 Known d



Compound

R<sup>1</sup> = CH<sub>2</sub>

R<sup>2</sup> = CH<sub>3</sub>

R<sup>1</sup> = CH<sub>3</sub>

R<sup>2</sup> = CH<sub>3</sub>

R<sup>1</sup> = CH<sub>2</sub>

R<sup>2</sup> = (CH<sub>3</sub>)

R<sup>1</sup> = CH<sub>2</sub>

R<sup>2</sup> = (CH<sub>3</sub>)

R<sup>1</sup> = CH<sub>2</sub>

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R<sup>2</sup> = (CH<sub>3</sub>)

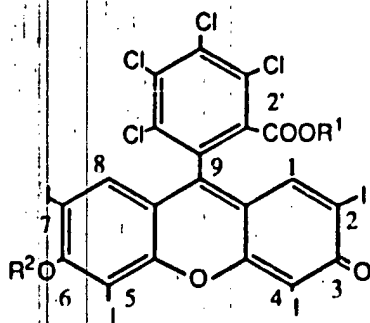
R<sup>1</sup> = CH<sub>2</sub>

R<sup>2</sup> = (CH<sub>3</sub>)

This typical spectrum is substantially blue shifted from the dye ionized at C-6 and has an order of magnitude lower  $\epsilon$  at its two  $\lambda_{\max}$ ,  $494 \pm 3$  nm and  $404 \pm 3$  nm. The shorter wavelength  $\lambda_{\max}$  at 404 nm is particularly important because it distinguishes the unionized form of the dye from the spectrum of the dimer. Substituting a carbon group at C-6 requires special conditions and, once the substituted C-6 compound has been prepared, special care in handling. Aside from many C-6 protonated forms of C-2' esters [11] and derivatives of the lactone of which the lactone diacetate is an example, there are only three known derivatives in which both C-2' and C-6 are covalent [13]. To the best of our knowledge there are no cases of pure compounds having been isolated in which C-2' is ionic and C-6 substituted. The one compound which has been reported, C-6-Me, C-2' carboxylate has the right sort of spectrum ( $\lambda_{\max}$  ( $\epsilon$ ) (EtOH) = 493 nm (5850), 402 nm (6610);  $\lambda_{\max}$  ( $\epsilon$ ) (EtOH/acetone) = 486 nm (5430), 394 nm (5930)) [14] but it has not yet been isolated as a pure solid. The spectra of the three disubstituted derivatives which have been isolated are given in Table 4.

TABLE 4

Known derivatives of Rose Bengal functionalized at C-6 with a covalently bonded group



Compound	$\text{CHCl}_3$ $\lambda_{\max}$ ( $\epsilon$ )	Acetone/EtOH (1:1)
$\text{R}^1 = \text{CH}_2\phi$ ; $\text{R}^2 = \text{CH}_3$	497 (12800) 403 (17900)	494 (21700) 403 (29100)
$\text{R}^1 = \text{CH}_3$ ; $\text{R}^2 = \text{CH}_3$	497 (11900) 403 (16900)	492 (17100) 402 (23700)
$\text{R}^1 = \text{CH}_2\phi$ ; $\text{R}^2 = (\text{CH}_3)_3\text{Si}$	497 (17400) 403 (24100)	494 (15800) 403 (22000)

To a greater or lesser degree the spectra of all C-6 ionic salts, whether they are substituted with a covalent group at C-2' or not, are comprised of a mixture of the spectra of the C-6 ionic and the C-6 covalent form. Differences in the spectrum of one compound with another result from secondary effects on the structure of the bare anion such as hydrogen bonding and dimerization due to hydrophobic dye/dye molecular interactions. This is particularly obvious when the fluorescence spectra of the ammonium salts at room temperature and at reduced temperature are

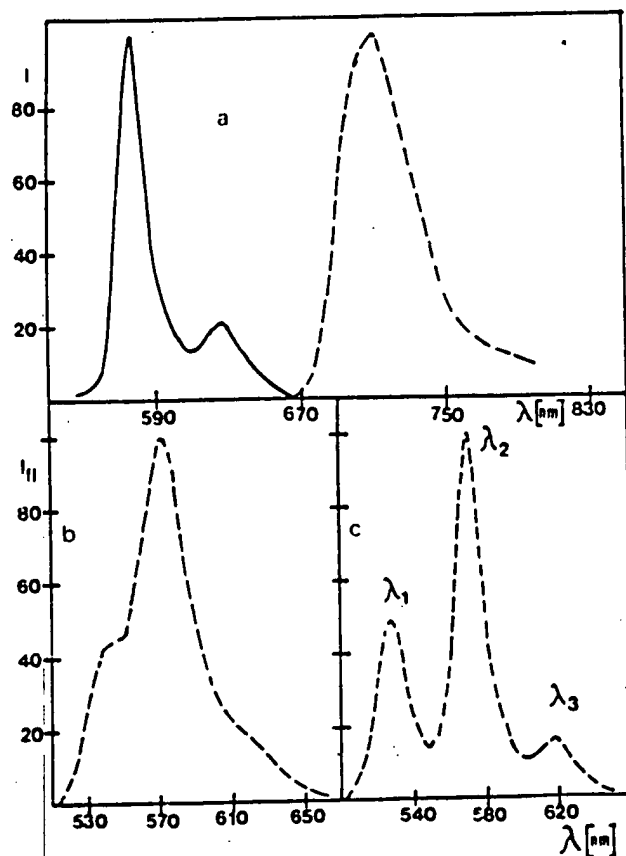


Fig. 2. Emission spectra of Rose Bengal derivatives. (a) Total emission spectra of Rose Bengal at 77 K. (b) Fluorescence emission of Rose Bengal, ditrimethylammonium salt in EtOH at ambient temperature. (c) Fluorescence emission of Rose Bengal, ditrimethylammonium salt in EtOH recorded at 77 K ( $\lambda_{\text{ex}} = 520$  nm).

compared (Fig. 2). Thus the fluorescence spectrum at room temperature of the C-2',C-6 bistrimethylammonium salt shows a broad triad of peaks centered at 583 nm (Table 3). At reduced temperature the same compound shows three sharper peaks at 538 nm, 583 nm and 605 nm (Fig. 2). The shortest wavelength of these emissions is due to the contact ion pair whereas the higher wavelength pair of peaks is the result of the totally dissociated dianion [15].

Specific examples illustrate that similar principles affect the absorption spectra. For example the C-2' benzyl ester, C-6 protio form is red in MeOH and orange in methylene chloride. Thus it has the spectrum of the ionized form in the polar, protic solvent and of the contact ion pair or unionized form in the non-polar solvent methylene chloride [6, 16] (Fig. 3(a)). The tributylammonium salt, C-2' octyl ester is soluble in solvents ranging from ethanol/water to toluene (Fig. 3(b)). Its spectrum, even in an essentially non-ionizing solvent such as toluene, is that of the ionized xanthene [17]. The spectrum of the pyrilium salt is concentration dependent. In dilute solution the compound is totally ionized and is red, whereas in concentrated

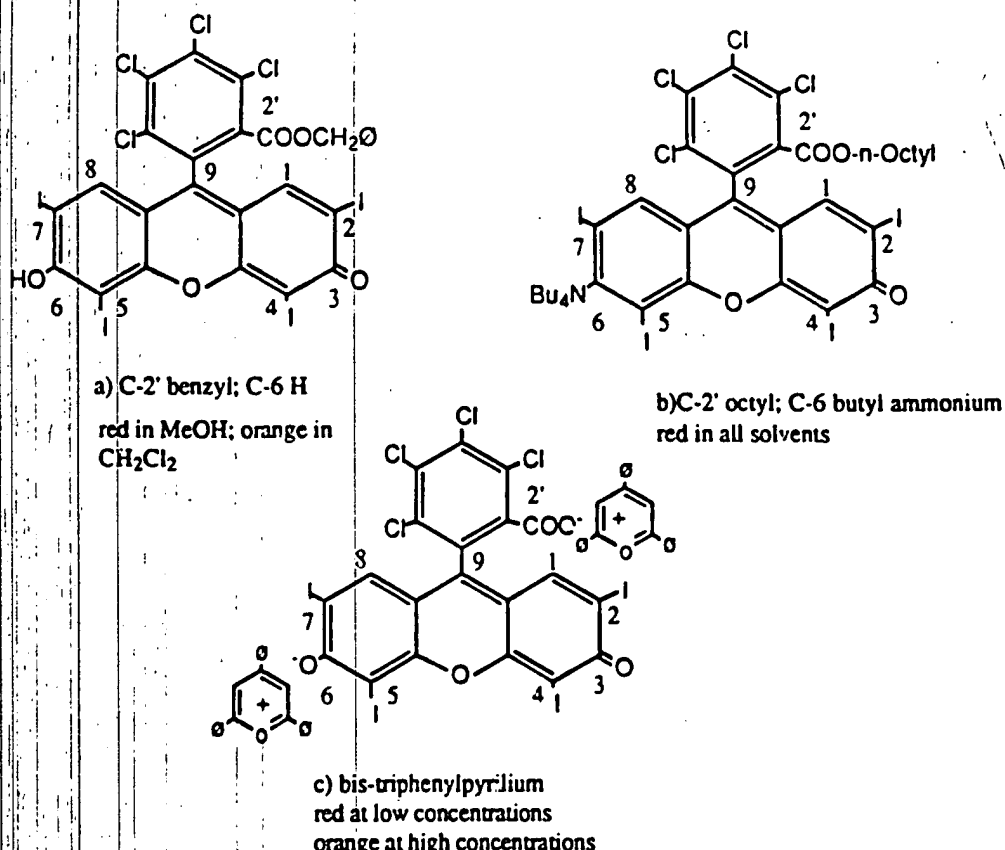


Fig. 3. (a) Rose Bengal, C-2' benzyl ester, C-6 H; (b) C-2' octyl ester; C-6 tetrabutylammonium salt; (c) C-2', C-6 bistrisphenylpyrylium salt.

solution the compound is not fully ionized and the orange form predominates. The relative degree of ionization in dilute solution being highest is merely an example of the law of mass action (Fig. 3(c)).

### 3.2. C-2' substitution

In solvents in which the group at C-6 is totally ionized, C-2' substitution has a much smaller effect on the spectrum than C-6 substitution. A subtle 5 - 7 nm shift to the red is observed in the  $\lambda_{\text{max}}$  relative to the dianion in virtually every solvent in which both are soluble. This results from the inability of C-2' to neutralize partially the zwitterionic C-6<sup>-</sup>, C-9<sup>+</sup> resonance contributor (Fig. 4). There is little difference in the extinction coefficients of Rose Bengal C-2' esters and the disodium salt.

### 3.3. Solvent effects

Solvent effects on the absorption spectra can be summarized as follows: if the compound is soluble in water, alcohols, and polar, non-protic solvents such as DMSO, DME and DMF, the  $\lambda_{\text{max}}$  increases in that order, i.e. it is most red shifted in polar, non-protic solvents. Compounds which are soluble in non-polar solvents such as  $\text{CH}_2\text{Cl}_2$  are generally not soluble

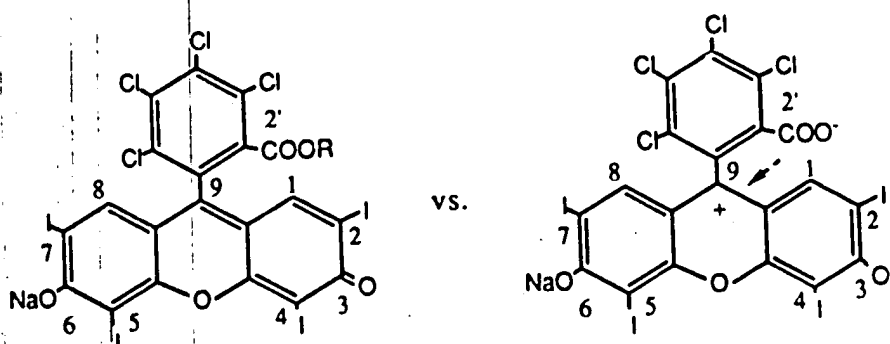


Fig. 4. C-2 esterification (left structure) prevents neutralization of the zwitterionic + charge at C-9. The C-2' esters absorb at slightly longer wavelengths than Rose Bengal, C-2', C-6 salts.

in water, and their absorption lies at about the same place in both alcohols and methylene chloride, but is shifted in polar, non-protic solvents to the red.  $\lambda_{\max}$  also traces the hydrogen-bonding ability of the solvent — the shorter the wavelength of the maximum absorption, the stronger the hydrogen bond to the solvent from the xanthene. The same is true for the fluorescence maximum. All of these generalizations are made clear by a careful examination of the data in Tables 2 and 3. The absorption spectra

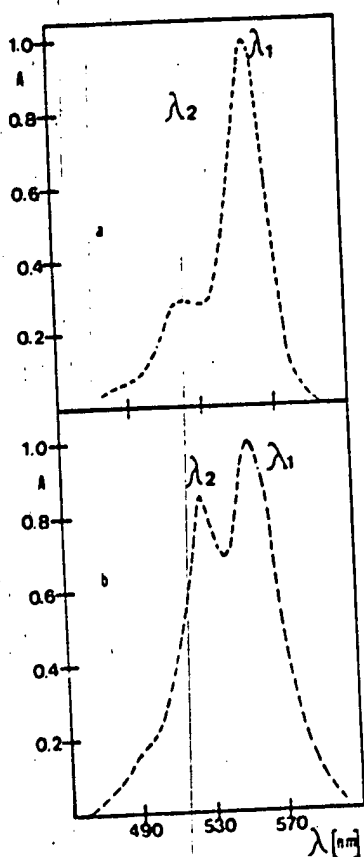


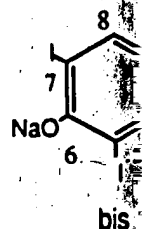
Fig. 5. Absorption spectra of (a) Rose Bengal in MeOH and (b) Rose Bengal, bis piperidinium salt, in  $\text{CH}_2\text{Cl}_2$ .

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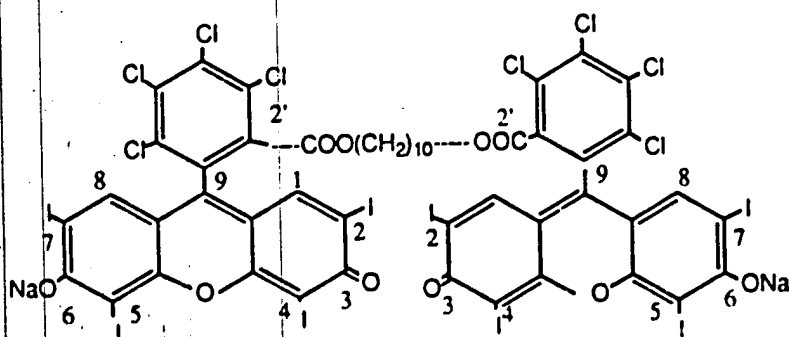


in methanol and methylene chloride (Fig. 5) indicates what solvation does to the appearance of the spectrum.

### 3.4. Aggregation effects

Rose Bengal, disodium salt, is soluble in water up to a concentration of approximately  $10^{-3}$  M. The spectrum follows Beer's law only below  $10^{-6}$  M whereupon subtle perturbations on the observed spectrum begin to appear. At higher concentrations the ratio of the two absorption maxima ( $\lambda_1/\lambda_2$ ) decreases because of the formation of aggregates, the latter being driven by hydrophobic effects [18]. Rose Bengal dimer has the absorption spectrum in water shown in Fig. 6 [19]. In contrast to the monomer, the dimer absorbs at the shorter wavelengths but goes through a minimum at the longer wavelengths. Therefore as the concentration of dimer increases the absorption at 550+ nm decreases whereas the absorption at  $525 \pm$  nm remains the same or increases slightly. This accounts for the marked change in ratio of the longer-wavelength absorption to that at shorter wavelength ( $\epsilon\lambda_1/\epsilon\lambda_2$ ) as a function of concentration in aqueous solution [20].

The effects of enforced aggregation have been studied in rationally synthesized dimers by Luttrull, Valdes-Aguilera, Linden, Paczkowski and Neckers [17]. The spectra of the Rose Bengal, C-2' ethyl ester, C-6 sodium salt and the decamethylene dimer, bis sodium salt



bis Rose Bengal, C-2' decamethylene, C-6 bis Na<sup>+</sup> salt.

are compared in Fig. 7. As is obvious from the comparison of the two spectra in Fig. 7, there is some broadening in the spectrum of the decamethylene dimer relative to the model. We have indicated that interaction between the two dye molecules leads to this perturbation in the normally sharp pair of peaks in the 525 - 555 nm region. This perturbation is a function of solvent and becomes particularly pronounced in water/ethanol of less than 20% EtOH where the very polar, protic solvent forces the non-polar organic Rose Bengals into a configuration which maximizes hydrophobic interactions. In comparing C-2' decamethylene esters in which C-6 is differently substituted, as with either a sodium ion or an organic cation, when C-6 is Na<sup>+</sup>  $\lambda_{\max}$  shifts to shorter wavelengths as the ethanol content is increased. When the cation at C-6 is organic,  $\lambda_{\max}$  shifts to shorter wavelengths initially as the EtOH content of the solvent is decreased, but

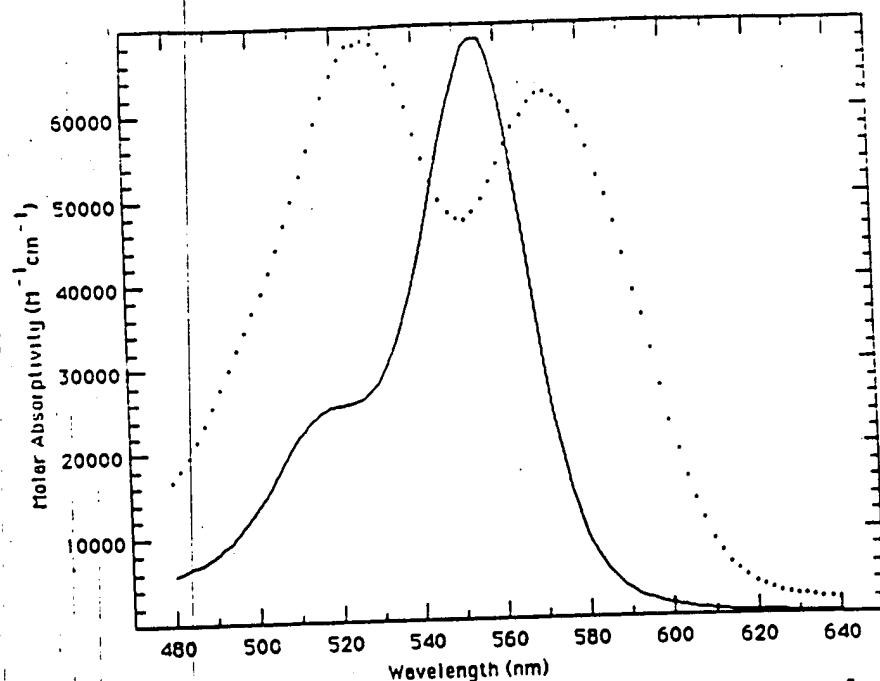


Fig. 6. Absorption spectrum of Rose Bengal, C-2' ethyl ester  $10^{-5}$  M in water (full line) and  $10^{-5}$  M in 1.0 M  $\text{KNO}_3$  (dotted line). The full line is the spectrum of the monomer; the dotted line is the spectrum of the dimer.

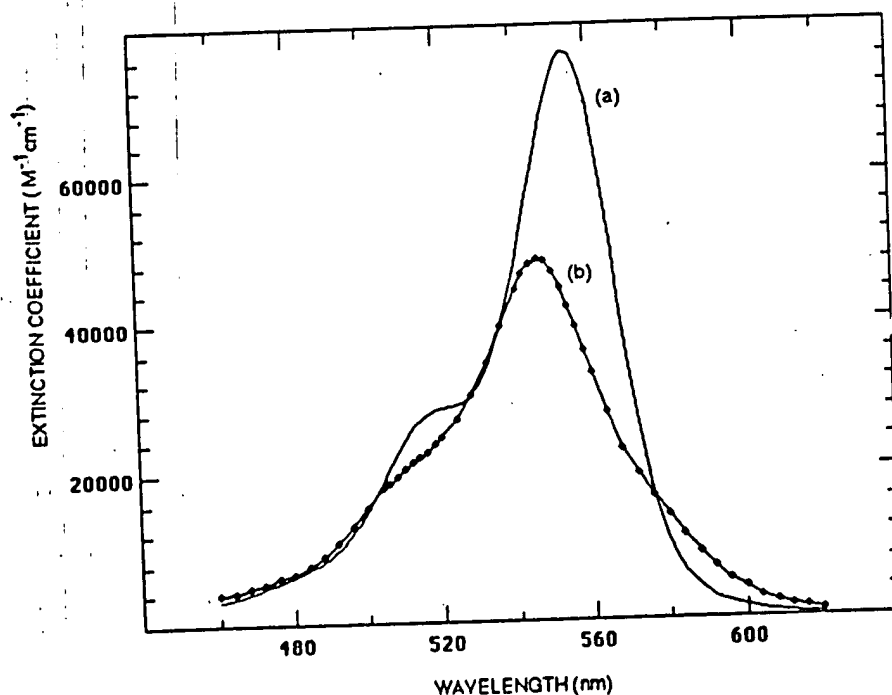


Fig. 7. Absorption spectra of (a) Rose Bengal, C-2' ethyl ester and (b) C-2'-decamethylene, C-6 bis  $\text{Na}^+$  salt.

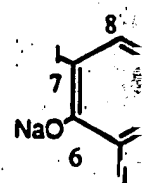
to longer wavelengths when the EtOH content of the solvent is below 50%. The extinction coefficient also changes as the EtOH concentration of the solvent is decreased, decreasing after the EtOH is reduced below 20%.

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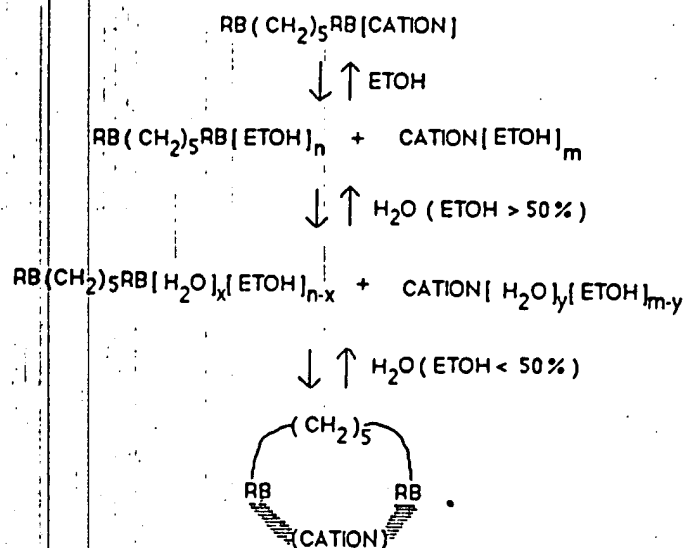
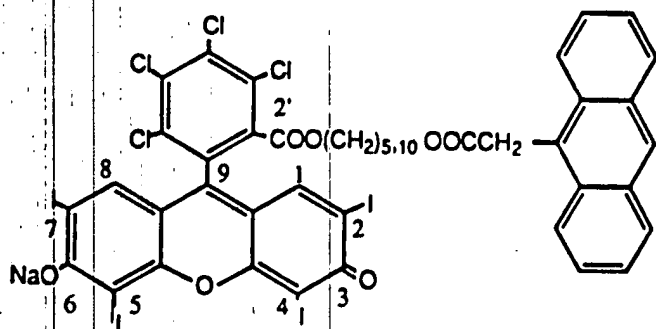


Fig. 8. Equilibria in ethanol for  $\text{RB}(\text{CH}_2)_5\text{RB}$  complex formation.

The presence of intramolecular aggregates is confirmed by comparing the absorption spectra of compounds such as the bis C-2' decamethyl ester and the excitation spectrum. We have proposed the equilibria outlined in Fig. 8 to explain these results.

The situation is similar with long-chain C-2' anthracene esters of Rose Bengal



The absorption spectra of two such compounds are given in Fig. 9. In the case with five methylene groups (I) there is perturbation in the Rose Bengal spectrum which is caused by the anthracene moiety positioned five carbons away. This perturbation on the Rose Bengal spectrum is more significant in the case of the decamethylene system (II). Based on the lack of emission from the aggregated form as shown by comparing the excitation and emission spectra, an equilibrium constant for the intramolecular complex [21] is calculated as 1.78 for the C-5 compound and 10.06 for the C-10. The triplet lifetime of only the decamethylene derivative (II) is lower than expected ( $\tau = 2.90 \mu\text{s}$  vs.  $8.0 \mu\text{s}$  for Rose Bengal, C-2' ethyl ester, C-6  $\text{Na}^+$  salt), though there is no evidence either of anthracene triplet being formed or of electron transfer products such as Rose Bengal radical anion.

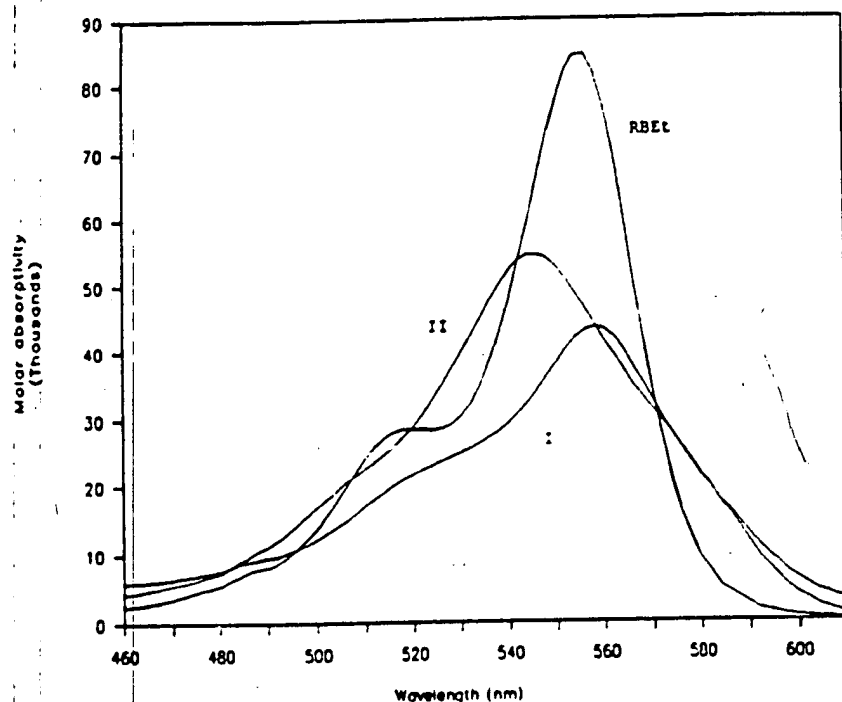


Fig. 9. Absorption spectra of Rose Bengal, C-2' pentamethylene anthranoate (I) and Rose Bengal, C-2' decamethylene anthranoate (II) compared with Rose Bengal, C-2' ethyl ester (RBET).

In polymeric systems the effects of proximity are threefold: effects on the absorption and emission spectra, effects on intermolecular energy transfer as measured by singlet oxygen yields, and intramolecular self-quenching. Spectral effects have been studied in two cases. In the first, poly(styrene-co-chloromethylvinylbenzenes) were treated with greater and greater concentrations of Rose Bengal in DMF [22]. In the second case, anionically polymerized styrenes were terminated with dibromoethane and the resulting  $\text{CH}_2\text{Br}$  end-capped polymer treated with Rose Bengal, producing linear polystyrene end-capped with Rose Bengal esters [23].

The effect of increasing the loading of Rose Bengal on a poly(styrene-co-chloromethylstyrene) on the absorption spectrum is, even in non-polar solvents such as methylene chloride, to produce results which can be attributed to aggregation. The absorption data for five poly(styrene-co-chloromethylstyrenes) differently loaded with increasing mg of Rose Bengal per gram of polymer are shown in Table 5.

The effects of loading on the absorption spectra in  $\text{CH}_2\text{Cl}_2$  solution and as a cast film are in the same direction but of different magnitude. We have interpreted this as meaning that the Rose Bengal moieties in the film are surrounded by the non-polar polymer whereas in  $\text{CH}_2\text{Cl}_2$  solvation effects predominate. There is a limited red shift as a function of dye concentration on the polymer backbone, but a major change in the relative extinction coefficients at the longer and shorter wavelengths. There is also

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TABLE 5

Absorption spectra of poly-RBs in  $\text{CH}_2\text{Cl}_2$  and in polymer films

Polymer <sup>a</sup>	$\text{CH}_2\text{Cl}_2$ solution			Thin films		
	$\lambda_1(\text{max})$	$\lambda_2(\text{max})$	$\epsilon_{\lambda_1}/\epsilon_{\lambda_2}$	$\lambda_1(\text{max})$	$\lambda_2(\text{max})$	$\epsilon_{\lambda_1}/\epsilon_{\lambda_2}$
poly-RB-51	571.0	523.0	3.16	576.5	534.5	2.19
poly-RB-102	572.0	532.0	2.68	578.0	537.0	1.83
poly-RB-152	572.0	532.0	2.42	578.0	537.0	1.79
poly-RB-450	572.5	532.0	1.94	577.5	537.0	1.72
poly-RB-610	572.5	532.0	1.59	578.0	536.5	1.63

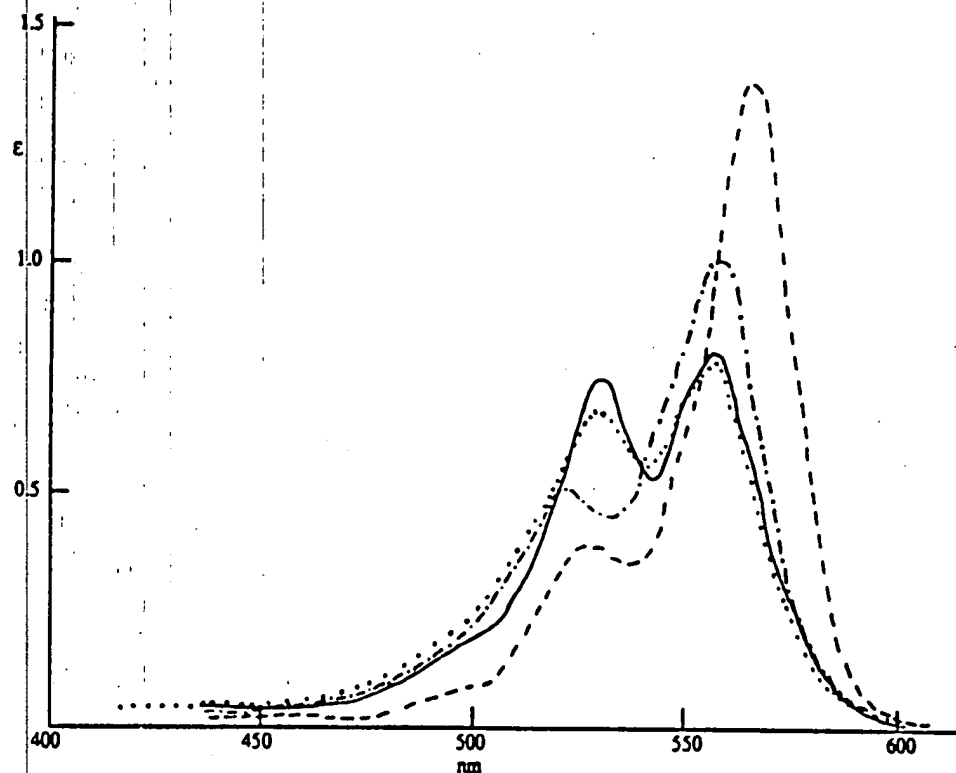
<sup>a</sup>mg Rose Bengal per gram of poly(styrene-co-chloromethylstyrene;3:1).

Fig. 10. Absorption spectra of polystyrene end-capped with Rose Bengal.

	$\lambda_{\text{max } 1}$	$\lambda_{\text{max } 2}$	$\epsilon_{\lambda_1}$ ( $\text{g}^{-1} \text{cm}^{-1}$ $\text{l}^{-1}$ )	$\epsilon_{\lambda_2}$ ( $\text{g}^{-1} \text{cm}^{-1}$ $\text{l}^{-1}$ )	$\epsilon_{\lambda_1}/\epsilon_{\lambda_2}$
— — — DMF	572	529	1.628	0.470	3.46
- - - $\text{CH}_2\text{Cl}_2$	562	523	1.167	0.587	1.99
..... Toluene	562	534	0.935	0.780	1.20
— — — Cyclohexane	562	534	0.932	0.870	1.07

an obvious broadening in the spectrum particularly at shorter wavelengths. We attribute these spectral changes to enforced aggregation of neighbor dyes on the polymer backbone. Unfortunately at the time these polymers were synthesized the fluorescence spectra were recorded only in thin films, and they were not accompanied by excitation spectra. The changes in spectra in the film can be attributed to self-absorption effects.

Rose Bengal end-capped polystyrenes show a marked change in spectrum as a function of solvent (Fig. 10) owing to differential solvating ability at C-6 in solvents of differing polarity. Though the polymers in Fig. 10 are identical, significant shifts in the spectra are observed from solvent to solvent.

Most interesting spectral changes occur with these polymers when they are irradiated in hydrocarbon solvents such as cyclohexane and toluene. When irradiated in these solvents the spectra of the polymers change in the direction of increased aggregation (Fig. 11). We have suggested that when irradiated the terminae of the polymers interact with the resulting product, which is dimeric at the ends rather than monomeric. Perhaps the driving force for this conformational change is electron transfer from one Rose Bengal terminus to another, followed by back electron transfer (Fig. 12).

Transient absorption spectra have been reported for certain intramolecular energy transfer events particularly in the case of anthracene derivatives of Rose Bengal [24], though their interpretation must be made with extreme caution. In MeOH, Rose Bengal C-2' ethyl ester undergoes bimolecular self-quenching with the appearance of three main absorption bands at 380, 465 and 605 nm 0.3  $\mu$ s after the flash. These bands decay

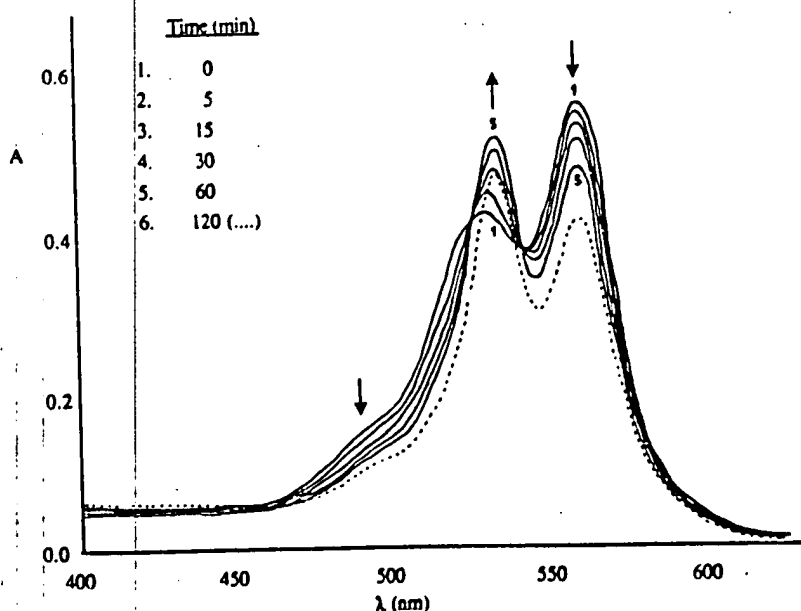


Fig. 11. Change in spectrum of polystyrene end-capped with Rose Bengal upon irradiation.

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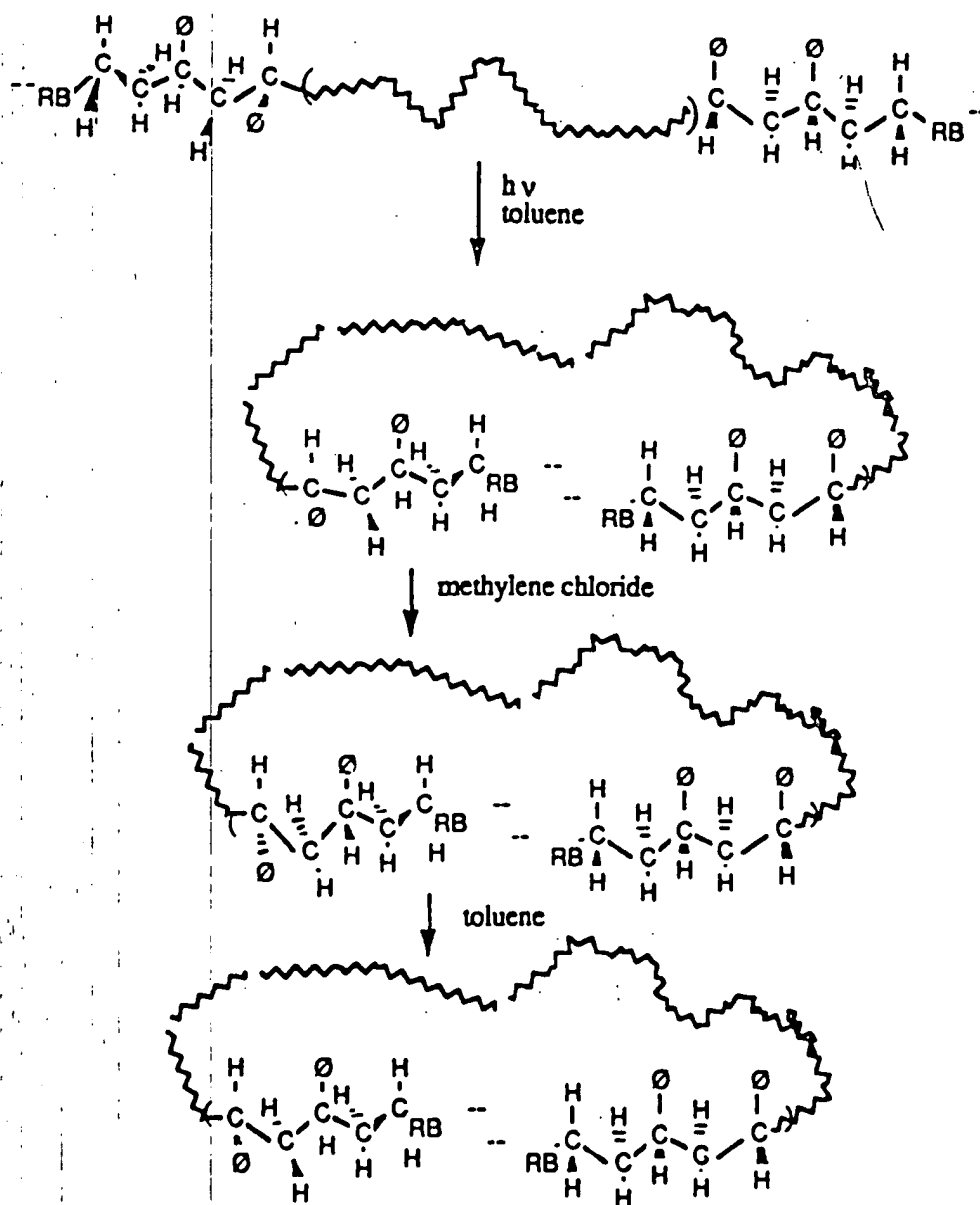


Fig. 12. Photochemically driven reorganization in polystyrenes end-capped with Rose Bengal.

in several hundred microseconds to bands at  $\approx 420$  nm and  $\approx 450$  nm respectively. Analysis of these transients shows that the triplet absorbs at  $> 600$  nm with  $\tau_T \approx 16$   $\mu$ s. This triplet lifetime is strongly dependent of RBET concentration in the concentration range  $(1 - 10) \times 10^{-5}$  M with a self-quenching rate constant of  $7.8 \times 10^7$   $M^{-1} s^{-1}$ . At shorter wavelengths the spectra are complicated by more than one species absorbing, but the peak at 420 nm grows in at a rate which is the same as the rate by which the triplet at  $\approx 600$  nm is being self-quenched. We suggest that this species is the radical anion. The radical cation grows in also at 450 nm [25].

#### 4. Quenching of Rose Bengal singlets and triplets: oxygen

The triplet of Rose Bengal, disodium salt, is "completely quenched" by oxygen in polar solvents producing singlet oxygen and superoxide radical ion. Discovered originally by Jodlbauer and von Tappeiner [26], it is this property more than any other which was responsible for the widescale use of Rose Bengal in the first place. Rate constants for oxygen quenching are at the diffusion-controlled limit under conditions of air saturation in all solvents. The details of this energy transfer have been described elsewhere but the quantum yields of singlet oxygen formation, fluorescence and intersystem crossing in MeOH at 20 °C are compared for four of the xanthenes in Table 6.

TABLE 6

Quantum yield of fluorescence, triplet formation and singlet oxygen formation for simple xanthenes<sup>a</sup>

Compound	$\phi_f$	$\phi_t$	$\phi(O_2)^b$
Rose Bengal, dianion	0.08	0.76	0.76
Erythrosin, dianion	0.08	0.62	0.62
Eosin, dianion	0.63	0.28	0.39
Fluorescein, dianion	0.93	0.03	0.09

<sup>a</sup>Data from Gollnick and Schenck [8]. Singlet oxygen reactions measured at [dye] =  $3.0 \times 10^{-4}$  using 2,5-dimethylfuran as the singlet oxygen trap; solvent MeOH at 20 °C.

<sup>b</sup>Quantum yields are a function of solvent, and are also likely a function of dye concentration.

Singlet lifetimes, fluorescence quantum yields and singlet oxygen quantum yields are compared for Rose Bengal, Erythrosin, Eosin and fluorescein in a number of different solvents in Table 7. In virtually every case water differs from the other solvents studied. Reference is made to Table 1 for additional data on Rose Bengal in water.

Singlet oxygen yields for several monomeric derivatives of Rose Bengal have been measured by Linden and Neckers. Because Rose Bengal, disodium salt, is only soluble in polar solvents certain of the oxidation products formed in singlet oxygen reactions may be consumed by these solvents in secondary reactions. Classic examples include 2,5-dimethylfuran [27] which produces the hydroperoxide hemiacetal rather than the subsequently isolated intermediate cyclobutadiene ozonide [28]. Singlet oxygen quantum yields from those Rose Bengal derivatives which have been reported are collected in Table 8 [29]. In polar solvents the values for each of the compounds do not vary much. In non-polar solvents, however, other processes which will be discussed later come into play.

Superoxide radical anion ( $O_2^{\cdot-}$ ) was first reported formed from Rose Bengal by Srinivasan *et al.* [30]. In a clever experiment  $O_2^{\cdot-}$  was detected

TABLE 7

Photophysical properties of simple xanthenes [8]; singlet lifetime as a function of solvent

$\tau \times 10^9 [\phi_f] [\gamma O_2]$

Compound



TABLE 7

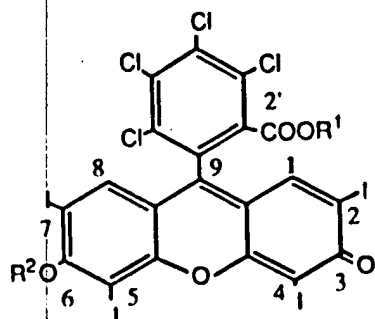
Photophysical properties of simple xanthenes [8]: singlet lifetime as a function of solvent—

 $\tau \times 10^9 [\phi_1] \{\gamma O_2\}$ 

Compound	<i>n</i> -BuOH	<i>n</i> -PrOH	EtOH	MeOH	Water
Rose Bengal dianion	0.77[0.14]{0.73}	0.71[0.13]{0.74}	0.69[0.11]{0.78}	0.44[0.08]{0.76}	0.05[0.01]
Erythrosin dianion	0.55[0.10]{0.65}	0.55[0.10]{0.65}	0.55[0.10]{0.65}	0.44[0.08]{0.62}	0.11[0.02]
Eosin dianion	3.15[0.68]{0.39}	3.21[0.71]{0.40}	3.34[0.72]{0.43}	2.92[0.63]{0.39}	0.51[0.11]
Fluorescein dianion	—	—	4.48[1.0]{0.07}	4.16[0.93]{0.09}	3.72[0.83]

TABLE 8

Singlet oxygen yields from derivatives of Rose Bengal [1, 2]



R¹, R²	$\phi O_2^a$ (MeOH)	CH₂Cl₂
$\phi_2I^+$ ; $\phi_2I^+$	0.66	b
$\phi CH_2$ ; $\phi_2I^+$	0.74	b
$\phi CH_2$ ; $\phi_3PCH_2\phi$	0.76	—
$\phi CH_2$ ; triphenylpyrilium	0.70	b
$\phi CH_2$ ; CH₃SO₂	0.73	—
$\phi_3PCH_2\phi$ ; $\phi_3PCH_2\phi$	0.74	—
Et; H	0.73	0.61
TEA; TEA	0.72	0.48
$\phi CH_2$ ; TEA	0.74	0.67
Et; TEA	0.74	0.71
Et; OAc	—	0.61
(CH₂)₇CH₃; TBA	—	0.40 (toluene)

<sup>a</sup>Data from S. M. Linden and D. C. Neckers, *Photochem. Photobiol.*, 47 (1988) 543.<sup>b</sup>In CH₂Cl₂ rapid bleaching via oxidative electron transfer precludes energy transfer. TEA = triethylammonium; TBA = tributylammonium.<sup>c</sup>Solvent in all cases MeOH; singlet oxygen trap is 2,3-diphenyl *p*-dioxene.

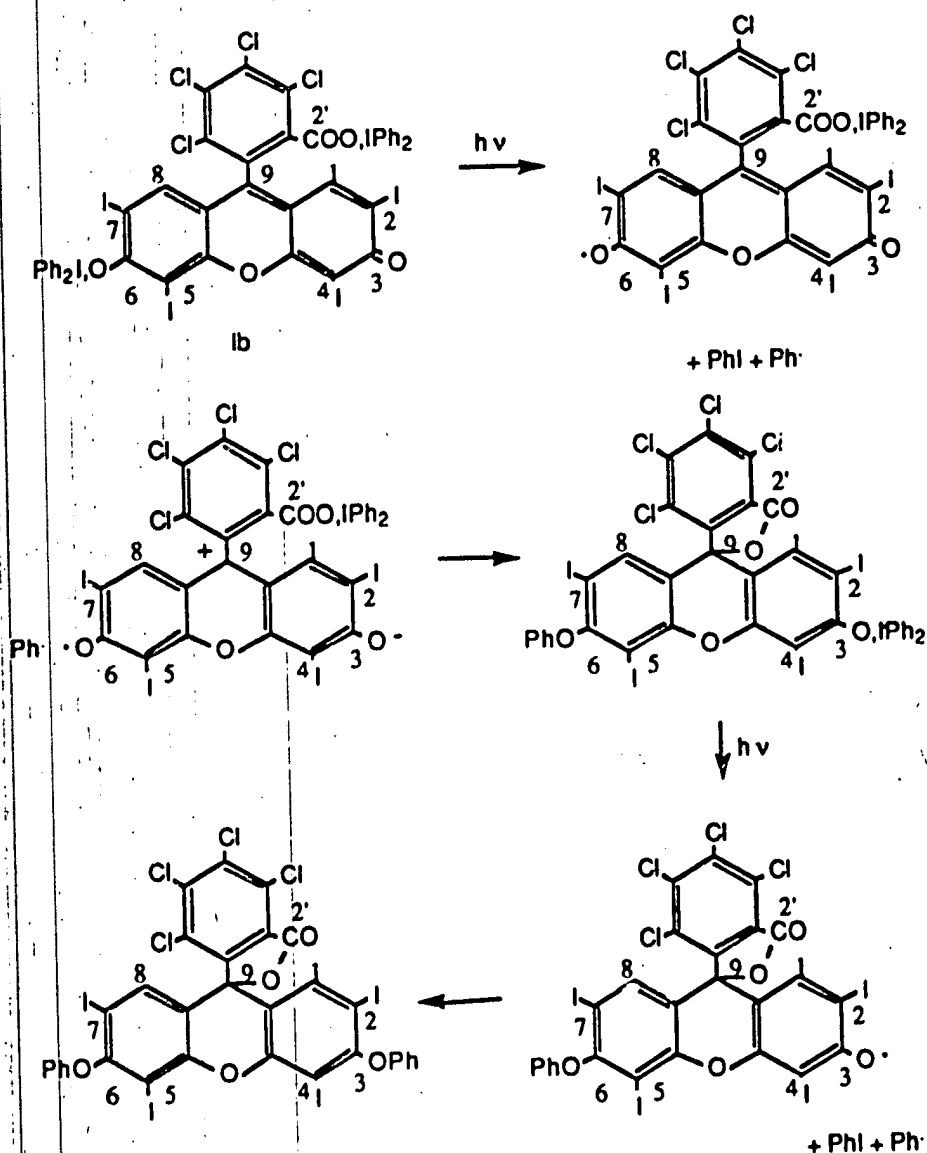
with superoxide dismutase and an oxygen electrode in water in the presence of sulfite. Rodgers and Lee later confirmed this result quantitatively [31] in water where 25% superoxide was observed. Superoxide formation may require something other than direct electron transfer from Rose Bengal triplet. The energetics allow self-quenching electron transfer followed by electron transfer from the radical anion of Rose Bengal to dioxygen. The mechanism, however, is bimolecular in Rose Bengal and suggests that the  $O_2^-$  yield should increase with the concentration of the dye. Though there are hints in the literature that this is the case [32], complete quantitative data on the yield of superoxide as a function of Rose Bengal concentration have not yet been reported [33].

### 5. Radical formation-dye oxidation and dye reduction

Eaton, in a review, reports that many xanthene dye reducing agents have been used as initiators of vinyl polymerization. Presumably these

are triplet reactions [34]. The oxidation and reduction potentials of those Rose Bengals which have been reported are given in Table 9.

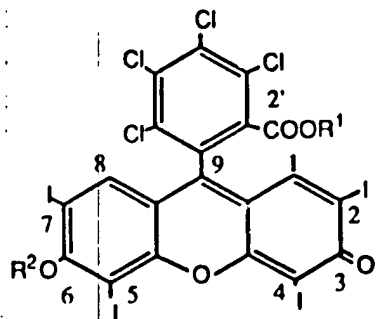
In non-polar solvents the iodonium salts, whether esterified at C-2' or not, bleach extremely rapidly. In each of these cases the gegen ion,  $\phi_2I^+$ , is easily reduced ( $\epsilon_{red} = 0.50$  eV) by the excited singlet state of the dye. Therefore the bleaching occurs by oxidative electron transfer from the dye to the gegen ion. Products deriving from this oxidation have been isolated and are shown below.



Methylene chloride solutions of the iodonium salts of Rose Bengal bleach from the singlet state in a few seconds in room light. The resulting phenyl radicals have been reported to initiate acrylate polymerization [35]. Other onium salts of Rose Bengal (Tables 2 and 3) bleach much more slowly and do so by a reductive mechanism.

TABLE 9

Oxidation and reduction of derivatives of Rose Bengal [34]



Compound; $R^1$ ; $R^2$	$E_{ox}^a$ (eV)	$E_{red}$ (eV)
$Na^+$ ; $Na^+$	0.86ev	-1.03ev
$\phi_2I^+$ ; $\phi_2I^+$	0.86	-1.00
$\phi_3PCH_2\phi$ ; $\phi_3PCH_2\phi$	0.85	-1.05
$Bu_4N^+$ ; $Bu_4N^+$	0.90	-1.01
$CH_2\phi$ ; $Na^+$	0.92	-0.905
$CH_2\phi$ ; $\phi_2I^+$	0.95	-0.895
$\phi CH_2$ ; $\phi_3PCH_2\phi$	0.93	-0.875
$CH_2\phi$ ; $CH_3SO_2^+$	0.94	-0.865
$CH_3$ ; $Na^+$	0.98	-0.86

<sup>a</sup>See notes in ref. 34 for experimental details.

The products of leuco Rose Bengal prepared under reducing conditions have been isolated by Zakrezewski [36]. Though reported by earlier workers to produce tetrachlorofluorescein [37] the primary photoreduction product of Rose Bengal is the dihydro compound (Fig. 13).

## 6. Conclusion

This review covers Rose Bengal chemistry, spectroscopy, photochemistry and photophysics. Because Rose Bengal has so many applications in biological and health sciences, a subsequent review will discuss the many and varied applications of Rose Bengal and its derivatives. Because Rose Bengal has been of specific interest to the author and his students, no attempt has been made to discuss the other xanthenes. In particular Eosin and fluorescein are extremely important dyes as well. These will also be the subject of a future review [38].

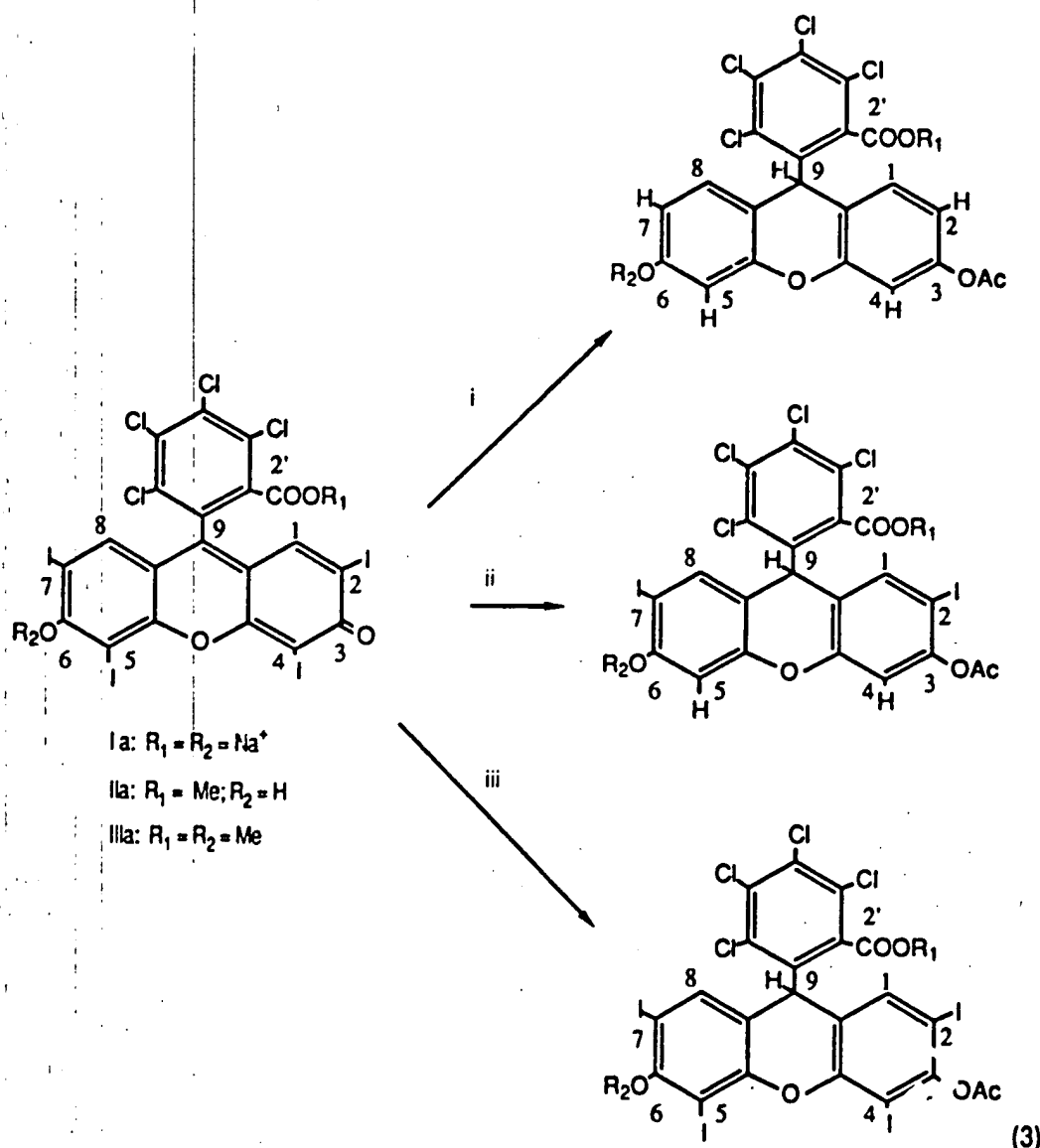


Fig. 13. Photoreduction of Rose Bengal under the following conditions. (i) (1) Zn, HOAc 24 h reflux; (2)  $\text{Ac}_2\text{O}$ , HOAc. (ii) (1) Zn, HOAc,  $60^\circ\text{C}$ , 24 h; (2)  $\text{Ac}_2\text{O}$ , HOAc. (iii)  $(\text{HOCH}_2\text{H}_4)_3\text{N}$ , DME, 4 h,  $h\nu$  [35]; (2)  $\text{Ac}_2\text{O}$ .

### Acknowledgment

This work has been supported by the National Science Foundation (Division of Materials Research and Chemistry Division) and the National Institutes of Health (National Cancer Institute). The author is extremely grateful for the continued support of these agencies. The author also recognizes the outstanding contributions of the many students and post-doctoral associates who have worked in his laboratories on this project over the years. Each of these individuals is named in the references to this review.

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38 The author would be pleased to receive from readers any and all information about any of the xanthenes, published or unpublished.